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# RP-HPLC Determination of Related substances of Pregabalin in bulk and pharmaceutical dosage form

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the determination of related substances in Pregabalin in bulk and pharmaceutical dosage form. The separation was carried out on a Inertsil ODS-3V $C_{18}$ column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of buffer, acetonitrile and methanol in a gradient elution at a flow rate of 0.8ml/min. The detection was made at 210 nm. The retention time of Pregabalin was found to be 6.5±0.1 min, amine amide impurity was found to be 28.8±0.1 min and lactam impurity was found to be 37.4±0.1min. Calibration curve was linear over the concentration range of 18.75-150 µg/ml of Pregabalin. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative estimation of related substances in drug and pharmaceutical dosage form.

Key words: Pregabalin, RP-HPLC, Phosphate Buffer, Inertsil ODS-3V C18 Column and Capsules.

## 1. INTRODUCTION

Pregabalin (S)-3-(amino methyl)-5-methyl hexanoic acid, it is an antiepileptic and structurally related to the inhibitory neurotransmitter amino butyric acid. It was recently approved in United states and Europe It was approved for treatment for partial seizers in adults[1,2]. Pregabalin is not official in any pharmacopoeia. Many reports are in literature for the estimation of Spectrophotometric[3],Charge-transfer complexes[4], LC-tandem mass spectroscopy[5],Simultaneous RP-HPLC[6],Human urine[7],RP-HPLC[8],Planar chromatograpy[9],HPLC-Fluorescamine[10].Since this drug is being marketed in domestic and international market the present investigation by the author describes a rapid, accurate and precise RP – HPLC method for the determination of related substances from bulk sample and pharmaceutical dosage form. The detector responses were linear in the concentration range of 18.75 – 150 µg/ml of drug and its related substances. The method was validated as per ICH guidelines.

## 2. EXPERIMENTAL

### 2.1. Chromatographic Conditions

Agilent 1200 series with high pressure liquid chromatographic instrument provided with a Inertsil-ODS 3V $C_{18}$ column (250 mm x 4.6 mm ; 5 $\mu$ ) Auto sampler, and VWD photo diode array detector, thermostatted column compartment connected with EZ Chrom software. HPLC grade methanol, acetonitrile , water were purchased from E. Merck Co; Mumbai, India, and diammonium hydrogen phosphate, ortho phosphoric acid AR grade were purchased from SD Fine Chem. Mumbai, India were used in the study.

### 2.2. Drug Samples

The reference sample and impurities supplied by Bio-Leo Analytical Labs India (P) Ltd, Prasanthinagar, Hyderabad. Branded formulation of Pregabalin was purchased from local market.

### 2.3. Mobile Phase

Accurately take 5.28g of diammonium hydrogen phosphate was weighed out and dissolved in 1000ml of water and adjust pH 6.5 with dilute phosphoric acid. For mobile phase preparation A, a mixture of buffer, methanol and acetonitrile in the ratio of 80:10:10 v/v, for mobile preparation B, a mixture of water and acetonitrile and water in the ratio of 10:90 v/v, the solutions were filtered through 0.45µ membrane filter and was degassed and Pregabalin and its impurities were eluted in a gradient program given in Table 1. Mobile phase was used as diluent for preparing the working solution of the drug and

pharmaceutical dosage form. The mobile phase was filtered through 0.45µ membrane filter and sonicated by using Biotechnics India Sonicator, Mumbai; the flow rate of the mobile phase was maintained at 0.8ml/min. The column temperature was maintained at 25ºC and the detection of the drug was carried out at 210nm.

Table -1: Gradient Programme

| Time(min) | Mobile phase **A** | Mobile phase **B** |
|---|---|---|
| 0 | 100 | 0 |
| 6 | 100 | 0 |
| 50 | 65 | 35 |
| 55 | 100 | 0 |
| 60 | 100 | 0 |

2.4. Preparation of standard stock solution

Weigh about 15mg of Pregabalin standard and transfers in to 50ml volumetric flask add about 30ml of diluent sonicate to dissolve resulting solution was diluted with the mobile phase.

2.5. Amine amide impurity stock solution

Weigh accurately about 15 mg of amine amide impurity into 50 mL volumetric flask. Add about 30 mL of diluent and sonicate to dissolve and further dilute to volume with diluent.

2.6. Reference Solution

Transfer 5 mL of standard stock preparation and 5 mL of Amine amide impurity stock solution into 20 mL volumetric flask dilute to volume with diluent.

2.7. Linearity and Construction of Calibration Curve

The quantitative determination of the drug was accomplished by a standard method. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working dilution of Pregabalin, Amine amide impurity and lactam impurity in the range of 18.75-150µg/ml were prepared by taking suitable aliquots of working standard solution in different 10ml volumetric flasks and diluting up to the mark with the mobile phase. 50µl quantity of the dilution was injected each time in to the column at a flow rate 0.8ml/min. Each dilution was injected 6 times in to the column. The drug elutes was monitored at 210 nm and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. The regression of the plot was completed by least squares regression method. A linear relationship in the range was found to the 18.75-150 µg/ml of the drug and its impurities between the concentration of analyte, and respective peak area. This regression equation was later used to estimate the amount of Pregabalin and its impurities in pharmaceutical dosage form. A representative chromatogram for the separation of Pregabalin and its impurities is given in fig.1

2.8. Sample Preparation

Weigh and transfer capsule powder equivalent to 300 mg of Pregabalin into 20 mL volumetric flask, add 15 mL of diluent, sonicate to dissolve for about 15 minutes and make up the volume with dilute. Further filter the solution through 0.45 micron filter.

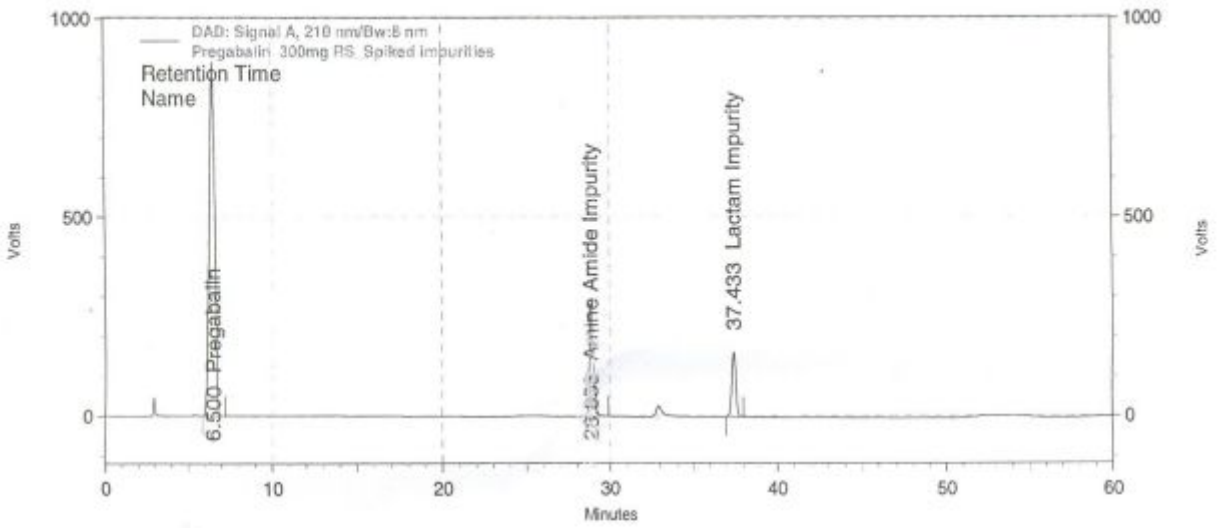
2.9. Placebo Preparation

Weigh and transfer placebo powder equivalent to 300 mg of Pregabalin into 20 mL volumetric flask, add 15 mL of diluent, sonicate to dissolve for about 15 minutes and make up the volume with dilute. Further filter the solution through 0.45 micron filter.

3. RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive precise and accurate HPLC method for the separation of Pregabalin in bulk drug and in pharmaceutical dosage form and forced degradation. In order to achieve optimum separation of the component peaks, mixtures of buffer with methanol and acetonitrile in different combinations were tested as mobile phase on a $C_{18}$ stationary phase. A binary mixture of buffer: methanol and acetonitrile in a gradient elution was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for Pregabalin was 6.1±0.1 for amide amine impurity was 28.9±0.1 min and for lactam impurity was 37.2±0.1min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of Pregabalin were reproducible as indicated by low coefficient of variation. A good linear relationship ($r^2$ = 0.9981) was observed for Pregabalin, $r^2$ = 0.9987 was observed for amide amine impurity and $r^2$ = 0.9998 was observed for lactam impurity the regression characteristics are given in table 2.

Fig -1: Chromatogram




DAD: Signal A, 210 nm/Bw:8 nm Results

| Name | Retention Time | Area | Peak purity |
|---|---|---|---|
| Pregabalin | 6.50 | 42714708 | 1.000000 |
| Amine Amide Impurity | 28.85 | 9596963 | 1.000000 |
| Lactam Impurity | 37.43 | 5577974 | 0.992548 |
| Totals | | 57889645 | |

Table -2 : Linearity of Pregabalin and impurities

| Linearity Level | Concentration (ppm) | Average Area of Pregabalin | Average Area of Amine amide impurity | Average Area of Lactam impurity |
|---|---|---|---|---|
| 25.0% | 18.75 | 433161 | 20652068 | 9414910 |
| 50.0% | 37.50 | 874095 | 41722488 | 21751267 |
| 100.0% | 75.00 | 1730992 | 82693148 | 42568879 |
| 150.0% | 112.50 | 2938254 | 138725458 | 62926713 |
| 200.0% | 150.00 | 3699890 | 180094869 | 85293800 |
| Correlation coefficient | | 0.9981 | 0.9987 | 0.9998 |

Table -3 : Recovery of Amine amide impurity

| Spike level | Amount Added (ppm) | Amount Recovered (ppm) | % Recovery | % Mean Recovery |
|---|---|---|---|---|
| LOQ level | 0.395 | 0.381 | 96.4 | 95.3 |
| LOQ level | 0.395 | 0.373 | 94.3 | |
| 25% | 19.763 | 21.633 | 109.5 | 109.5 |
| 25% | 19.763 | 21.628 | 109.4 | |
| 50% | 39.525 | 40.072 | 101.4 | 101.3 |
| 50% | 39.525 | 40.044 | 101.3 | |
| 100% | 79.050 | 73.203 | 92.6 | 92.6 |
| 100% | 79.050 | 73.182 | 92.6 | |
| 150% | 118.575 | 128.362 | 108.3 | 108.2 |
| 150% | 118.575 | 128.303 | 108.2 | |
| 200% | 158.100 | 168.086 | 106.3 | 106.3 |
| 200% | 158.100 | 168.186 | 106.4 | |

Table -4: Recovery of lactam impurity

| Spike level | Amount Added (ppm) | Amount Recovered (ppm) | % Recovery | % Mean Recovery |
|---|---|---|---|---|
| LOQ level | 0.351 | 0.360 | 102.7 | 103.9 |
| LOQ level | 0.351 | 0.368 | 105.0 | |
| 25% | 17.538 | 17.899 | 102.1 | 102.2 |
| 25% | 17.538 | 17.942 | 102.3 | |
| 50% | 35.075 | 36.567 | 104.3 | 104.2 |
| 50% | 35.080 | 36.542 | 104.2 | |
| 100% | 70.150 | 67.268 | 95.9 | 95.7 |
| 100% | 70.150 | 67.060 | 95.6 | |
| 150% | 105.225 | 96.510 | 91.7 | 92.0 |
| 150% | 105.225 | 97.156 | 92.3 | |
| 200% | 140.300 | 131.447 | 93.7 | 93.2 |
| 200% | 140.300 | 129.946 | 92.6 | |

Table -5: Robustness study

| Condition | Resolution | % Impurities | | % Difference | |
|---|---|---|---|---|---|
| | | Lactam Impurity | Total | Lactam Impurity | Total |
| Normal Condition (i.e As such condition) | 35.04 | 0.001 | 0.001 | NA | NA |
| Column Temperature changed to 30°C | 29.75 | 0.001 | 0.001 | Nil | Nil |
| Column Temperature changed to 20°C | 63.30 | 0.003 | 0.003 | 0.002 | 0.002 |
| Flow rate changed to 1.0 mL | 29.91 | 0.001 | 0.001 | Nil | Nil |
| Flow rate changed to 0.6 mL | 27.94 | 0.001 | 0.001 | Nil | Nil |

Table -6: LOD Study

| Name of the Component | S/N Ratio | % level of component w.r.t to sample concentration | Value (mcg/ml) |
|---|---|---|---|
| Pregabalin | 3.38 | 0.01 | 10 |
| Amine amide impurity | 3.06 | 0.001 | 1 |
| Lactam impurity | 2.92 | 0.0015 | 1.5 |

Table -7: LOQ Study

| Name of the Component | S/N Ratio | % level of component w.r.t to sample concentration | Value (mcg/ml) |
|---|---|---|---|
| Pregabalin | 9.99 | 0.020 | 20 |
| Amine amide Impurity | 9.99 | 0.002 | 2 |
| Lactam impurity | 9.65 | 0.003 | 3 |

Table -8 **:** Solution stability

| Time (hours) | % of Lactam impurity | % of difference | %Total impurities | % of difference |
|---|---|---|---|---|
| 0 (Initial) | 0.001 | Nil | 0.001 | Nil |
| After 24 hours | 0.001 | Nil | 0.001 | Nil |
| After 48 hours | 0.001 | Nil | 0.001 | Nil |

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The impurity content in capsules was quantified using the proposed analytical method are given in table 3 and 4.

The percentage of individual and total impurities observed were deliberate changes in the method proves that the method is robust. The robustness study results are presented in table 5. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The results are presented in Table 6 and 7. The difference between initial and bench top stability sample for % of individual impurities and total impurities were found within the acceptance criteria which indicates the solution were stable up to 48 hours. The results are presented in table 8.

The precision was established by six replicate injections at LOQ level of the test preparation containing impurities of interest. The values of relative standard deviation were found to be 1.56 and 2.13 within the acceptance limit, indicating the injection repeatability of the method. The results are presented in Table 9.

Table -9 : Precision study

| Amine amide impuri | Lactam impurity |
|---|---|
| 439383 | 235863 |
| 429742 | 241116 |
| 430631 | 240618 |
| 437916 | 235429 |
| 447665 | 228964 |
| 432343 | 230521 |
| 436280 | 235419 |
| 6812.9 | 5006.8 |
| 1.56 | 2.13 |

The specificity of the HPLC method was determined by the complete separation of impurities with Pregabalin. It was observed that there was no interference of blank and placebo at the retention time of analyte and impurity peaks. Peak purity of analyte and individual impurities should not be less than 0.99 the results of specificity data for degradation study are given in table 9.

Table 10: Specificity study

| Test Preparation name | Peak Purity |
|---|---|
| Pregabalin | 1.0000 |
| Amine amide impurity | 1.0000 |
| Lactam impurity | 1.0000 |

The intermediate precision (ruggedness) of the method was by carried out precision study in six preparations of a sample in a single batch sample by two different analysts, on two different columns and on two different instruments was found to be within the acceptance limit, which shows that the method is rugged. The results are presented in Table 10.

Table -10 : Intermediate Precision (ruggedness) study

| S. No | Lactam impurity areas |
|---|---|
| 1 | 17572 |
| 2 | 17958 |
| 3 | 16554 |
| 4 | 16967 |
| 5 | 17491 |
| 6 | 18257 |
| Avg: | 17467 |
| SD: | 626.2 |
| % RSD: | 3.58 |

The filter paper variation of the method was carried out by injected filtered through different 0.45 µ membrane filters, the difference between % of individual and total impurities were found within the acceptance limit. The results are presented in Table 11.

Table -11 : Filter Variation Study

| % of Lactam impurity in test solution with 0.45µ Nylon filter | % of Lactam impurity in test solution with 0.45µ PVDF filter | % Difference |
|---|---|---|
| 0.001 | 0.001 | Nil |
| % of Total impurities in test solution with 0.45µ Nylon filter | % Total impurities in test solution with 0.45µ PVDF filter | % Difference |
| 0.001 | 0.001 | Nil |

## 4. CONCLUSION

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the determination of related substances in Pregabalin and in pharmaceutical dosage form.

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